

Soil drying and nitrogen availability modulate carbon and water exchange over a range of annual precipitation totals and grassland vegetation types

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Abstract

Increased intensity in precipitation events and longer periods of water deficit are predicted as a general trend under future climate scenarios with potentially large effects on terrestrial ecosystem function. The primary objective of this study was to understand how variation in the intensity of precipitation inputs followed by intermittent soil drying events influence leaf and ecosystem carbon dioxide (CO₂) and water exchange in a California annual grassland mesocosm experiment. We further examined how nitrogen (N) availability, and differences in plant community composition (grass-forb combinations) affected gas exchange responses to the precipitation treatments. Net ecosystem CO₂ exchange (NEE) and evapotranspiration (ET) increased significantly with greater precipitation and were positively correlated with soil moisture. A repeated 10-day soil drying period, following 11 days of watering, strongly depressed NEE over a range of annual precipitation totals (297, 657 and 987 mm), and plant community types. Ecosystem dark respiration (R_e) and leaf level photosynthesis (A_{max}) showed greater sensitivity to periods of soil drying in the low precipitation plots (297 mm). N additions significantly increased NEE and R_e , particularly as water availability was increased. Across the range of precipitation totals and plant community types, intermittent periods of soil moisture deficit and native soil N availability constrained leaf and ecosystem level CO₂ exchange, while the influence on water vapor exchange was less pronounced.

Keywords: climate change, drought, ecosystem, fluxes, forbs, gas exchange, grassland, photosynthesis, precipitation, pulse, respiration, transpiration

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Introduction

Human activities are simultaneously altering global temperature, atmospheric carbon dioxide (CO₂) concentrations, nitrogen (N) deposition rates and precipitation patterns (IPCC, 2007). These changes have the potential to drastically change plant function and ecosystem processes at a global scale (Nemani *et al.*, 2003). Precipitation patterns are a defining characteristic of earth's biomes (Gurevitch *et al.*, 2006) and are a primary controller of ecosystem composition and function (Knapp & Smith, 2001). Soil moisture integrates how biological systems

respond to climate change variables, such as rising CO₂ and temperature, and changes in precipitation patterns (Weltzin *et al.*, 2003). For example, research shows that the effects of elevated CO₂ and N deposition on plant productivity vary considerably depending on soil moisture conditions (Schimel *et al.*, 1997; Poorter & Perez-Soba, 2001). One of the major influences of warming on ecosystem function occurs through soil moisture loss with increased rates of evapotranspiration (ET) (Harte *et al.*, 1995; Calanca *et al.*, 2006).

Grasslands have been used as model systems to understand ecosystem responses to human alterations of global resource cycling and climate change (Field *et al.*, 1996). Grasslands are particularly responsive to variability in precipitation (Knapp & Smith, 2001).

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Several studies conducted in California annual grasslands have demonstrated neutral to positive effects on productivity due to increases in annual precipitation (Dukes *et al.*, 2005; Harpole *et al.*, 2007b; Suttle *et al.*, 2007). Far fewer studies have focused on California grassland responses to water deficits because it is difficult to control ambient precipitation in field studies. However, recent climate models suggest that California and much of the subtropics, where grasslands are abundant, are more likely to experience increases in drought during the growing season (Hayhoe *et al.*, 2004; IPCC, 2007).

Mean annual precipitation is an important determinant of grassland productivity (Knapp & Smith, 2001), but there is good evidence that variation in intra-annual precipitation patterns also significantly influences grassland function and productivity. For example, in a perennial grassland, Knapp *et al.* (2002) showed reductions in annual net primary productivity by increasing the duration of the dry periods between more intense rainfall events, while holding total rainfall constant. Reduction in productivity was partially attributed to more frequent and prolonged periods of soil moisture deficit associated with increased variability in soil water content (Knapp *et al.*, 2002). Understanding the effects of precipitation patterns on grassland function is important as climate models project that intra-annual precipitation variability will increase in the future (Easterling *et al.*, 2000; Groisman *et al.*, 2005; Sun *et al.*, 2007).

N is the element that most commonly limits biological productivity of temperate terrestrial ecosystems (Vitousek, 2004), including grasslands (Harpole *et al.*, 2007a; LeBauer & Treseder, 2008). N inputs into the global N cycle have doubled as a result of human activity and are predicted to continue rising (Vitousek *et al.*, 1997a; Gallo-way *et al.*, 2004). Ecological consequences of human alteration of the global N cycle include soil acidification, increases in stored terrestrial carbon and loss of species diversity (Vitousek *et al.*, 1997b; Fenn *et al.*, 2003a). The size and flux of soil N pools, as influenced by atmospheric inputs (Fenn *et al.*, 2003b), microbial activity (Stark & Firestone, 1995), and losses are largely determined by precipitation events (Schimel *et al.*, 1997). It is therefore critical to understand how ecosystem responses to N availability may be affected by shifts in the timing and intensity of intra-annual precipitation events.

Measures of CO₂ and water (H₂O) exchange can provide an integrated view of ecosystem water relations and carbon metabolism. Studies characterizing ecosystem CO₂ and water vapor flux responses to pulses of water have been conducted in annual (Harpole *et al.*, 2007b) and semiarid grasslands (Huxman *et al.*, 2004; Potts *et al.*, 2006; Patrick *et al.*, 2007). However, a better understanding of how intermittent periods of water

deficit influence ecosystem processes is lacking. Using a high precision watering system in a mesocosm experiment we examined the effects of variation in annual precipitation totals, soil drying, N availability and plant community composition on annual grassland CO₂ and H₂O fluxes. We tested the following hypotheses: (1) leaf level and ecosystem CO₂ and H₂O exchange rates increase concomitantly with precipitation; (2) intermittent periods of soil drying negatively affect grassland gas exchange rates, particularly when cumulative water status is low; (3) greater precipitation increases responsiveness to N; (4) plant community composition and structure influences sensitivity to precipitation and N inputs.

Materials and methods

Soil and seed collection

In the spring of 2005, soil was collected at the Hopland Research and Extension Center which is located in Mendocino County in the Coast Range of northwest California. The site was prepared for soil collection by removing native vegetation. The soil was excavated by horizon, using a grader, and transported to Richmond, CA where the experiment was conducted. The soil was classified as a Typic Haplustept, with A, B1 and B2 horizons each of which were on average 20 cm thick. Field bulk densities of 1.15, 1.4, and 1.6 g cm⁻³ for the A, B1 and B2 horizons, were determined. Each horizon's soil was then sieved to roughly 8 mm using a large screen. Seeds for all species were also collected at the Hopland Research and Extension Center in June of 2005 and 2006, in the vicinity of the soil collection site.

Plant growth conditions

Mesocosms, 57 cm in diameter and 66 cm tall, were constructed from thick wall polyvinyl chloride (PVC). A screen and fine sand were placed at the bottom of the mesocosm above a drainage hole to ensure adequate drainage. In the mesocosms, the native soil profile was reconstructed by packing each 20-cm horizon in its original position. To achieve the target bulk density we put the same mass of a given horizon soil into each mesocosm, and packed it to the correct volume. Two 10 mm pulses of water were applied to the soil surface of each mesocosm to induce germination of the native seed bank; all germinated plants were removed, allowing control of plant community composition. Following native vegetation removal the soils were then allowed to air dry in the greenhouse for several weeks before initiation of the experiment.

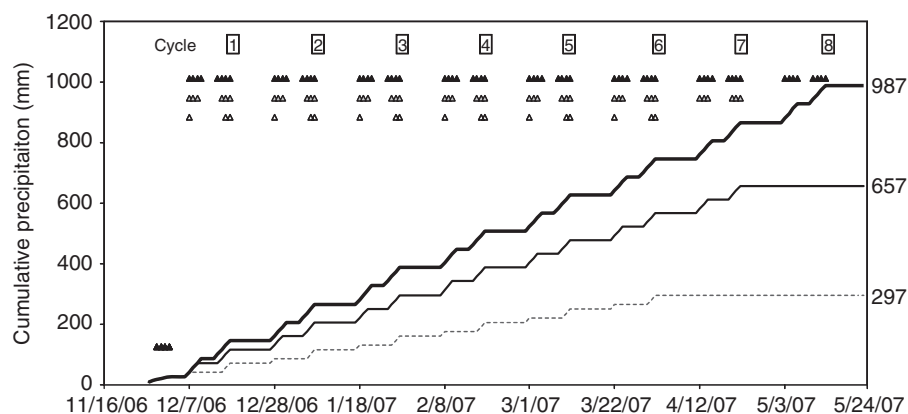


Fig. 1 2006–2007 precipitation regime. Within the 11 day watering period at the beginning of each of the 21 day water cycles the high ambient and low precipitation plots received eight, six and three 15 mm precipitation events respectively (each triangle symbol at the top of the graph represents a 15 mm irrigation event). Each water treatment then experienced a uniform soil drying period of 10 days at the end of each 11 day watering period within each water cycle. Cumulative season precipitation totals for each of the three treatments were 987, 657 and 297 mm, respectively.

The experiment was conducted in a climate-controlled greenhouse in Richmond, CA (37°54'50"N, 122°19'40"W). We used an unreplicated, randomized complete block design with three main treatment factors in five blocks. The treatments included three different plant communities, three watering levels and two N levels. The first vegetation type was a mixed grass-forb community consisting of five grasses and two forbs sown at the following density (seeds m^{-2}): *Avena barbata* (1500), *Bromus hordeaceus* (1500), *Briza maxima* (1000), *Hordeum murinum* (1000), *Aegilops triuncialis* (500), *Erodium botrys* (250) and *Amsinckia douglasiana* (250). The second and third community types were *Avena* and *Erodium*, which are a dominant grass and forb species, grown in monoculture communities at densities of 4000 and 2000 seeds m^{-2} . Sowing densities and species proportions were based on composition and density studies conducted in the vicinity of the Hopland Research and Extension Center (Pitt & Heady, 1978; Corbin *et al.*, 2007).

The experiment was conducted for two growing seasons (2005–2006, 2006–2007) beginning with seed germination in late November and ending with plant senescence in mid May. In November of 2005, all mesocosms received a uniform amount of water (45 mm of water over an 11 day period) before imposing the precipitation treatment so that seed germination would be uniform. In the 2006 soil-wetting period, each mesocosm received 27 mm of water over a 7-day period. During the 2005–2006 season, precipitation treatments (annual totals) included dry (315 mm yr^{-1}), ambient (675 mm yr^{-1}), and wet (1245 mm yr^{-1}) conditions, representative of the annual precipitation totals observed during average and dry and wet years at Hopland Research and Extension Center from 1989 to 2005. During the 2006–2007 season

the three precipitation treatments were adjusted slightly to 297 mm yr^{-1} , 657 mm yr^{-1} , and 987 mm yr^{-1} .

The pattern of water application that defined the precipitation treatments over the course of the growing season was determined based on an analysis of climate data from the Hopland station. We found that variation in total rainfall among years was primarily due to the number of rainy days, the intervals between rain events, and the duration of the season, whereas the average precipitation per rainy day did not vary between dry and wet years. According to this pattern, each 1-day watering event was held constant at 15 mm. Cumulative annual precipitation differences that define the precipitation treatments were the result of differences in the number of 15 mm precipitation events [high precipitation = 10 (2005–2006) or 8 (2006–2007), ambient = 6, low = 3] applied within the 11 day watering period in the 21 day repeating water cycle (Fig. 1). The precipitation treatments were applied using an automated watering system designed to uniformly apply precise amounts of water across the soil surface during each irrigation event. Computer-controlled electronic relays operated programmable water pumps and a series of solenoids to precisely fill the water reservoirs associated with each mesocosm. Irrigation tubing connected the water reservoirs to two concentric rings of drip irrigation tubing on the soil surface of each mesocosm, through which water was slowly released.

In May of 2006, aboveground plant biomass and seeds were harvested. In the summer of 2006 the mesocosms were reseeded as explained above and 100 g of homogenized plant biomass without seeds (within each of the three vegetation types) was added as litter. One hundred grams represented about 35%

of the total aboveground biomass produced in each of the vegetation types during the 2005–2006 growing season. N was added in the form of ammonium nitrate (dissolved in water) and applied at a rate of 2 g N m^{-2} , four times during the spring of 2007 (during water cycles 4–6) for a total of 8 g N m^{-2} . Leaf and whole-mesocosm gas exchange, canopy height, leaf area index (LAI) and soil moisture were measured at the end of the 11-day wet period (wet max) and 7 days into the dry period during each water cycle over the 2006–2007 season.

Soil moisture and environmental sensing

Soil moisture in the mesocosms was monitored with horizontally installed 0.30 m time-domain reflectometer (TDR) probes, calibrated to provide the relationship between the dielectric constant (measured by the TDR probe) and the corresponding volumetric soil moisture content (SMC). A dielectric-SMC relationship was determined separately for each of the three soil horizons. During the first year of the experiment the TDR probes were located at 0.10 m (center of the A horizon) and 0.50 m (center of the B₁ horizon). Before the start of the second year, an additional TDR probe was installed to monitor SMC at 0.25 m depth (center of the B₂ horizon). Environmental sensors and a data logging system were used to measure and record air temperature, humidity and light intensity in 15 min intervals. During the 2007 growing season mean temperature in the greenhouse ranged from 10 to 19 °C with an average of 15 ± 2.4 °C. Relative humidity ranged from 48% to 88% with an average of $70 \pm 8.7\%$. A lighting system with high-pressure sodium bulbs was used to balance day-time light gradients. Photosynthetic photon flux density (PPFD) varied throughout the day with a maximum of $1800 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and an average of $701 \pm 322 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Ecosystem CO₂ and H₂O exchange measurements

A portable, transparent flux chamber enclosing an infrared CO₂ and H₂O analyzer (IRGA; Licor 7500, Licor Environmental, Lincoln, NE, USA) was used to measure ecosystem (whole-mesocosm) CO₂ and water vapor fluxes (Patrick *et al.*, 2007). Positive seating of the chamber on the mesocosm was accomplished with a bottom plate with a foam gasket and side walls that fit on the top of the PVC rim. The IRGA and a mixing fan were mounted to the chamber cover. The chamber height was increased by adding a second segment as the plants grew. Each chamber segment was 54 cm tall, and was comprised of circular top and bottom plates joined by eight rods. High transparency (>95% for

photosynthetically active radiation), 50- μm -thick fluorocarbon plastic film (Dupont FEP, Wilmington, DE, USA) was used to cover the sides and top of the chamber. Using a flexible plastic film minimized soil–atmosphere pressure gradients that can dampen flux rates (Saleska *et al.*, 1999). The importance of any small leaks in the chamber was estimated by introducing a gradient of 400 ppm CO₂ between the chamber and room air and measuring the loss of CO₂ from the chamber when the chamber was placed on a flat surface containing no soil or vegetation; the results were insignificant compared with typical ecosystem fluxes (i.e., $<0.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

Changes in CO₂ and H₂O concentrations in the chamber over a 40–60 s interval were used to determine rates of net ecosystem CO₂ exchange (NEE), and ET. Ecosystem dark respiration (R_e) was measured by placing a dark cloth over the chamber and repeating the measurement. Measurements were made during the day which gives a measure of ecosystem dark respiration but is not a direct measure of night-time respiration. Change in concentration with time, dC/dt ($\text{mmol CO}_2 \text{ cm}^{-3} \text{ s}^{-1}$), was obtained by fitting a linear regression of concentration on time after removing the first 10 s of the measurement to allow for the instrument to settle and air to mix in the chamber. Typically, the linear fit captured between 80% and 95% of the variance in concentration, allowing dC/dt to be determined to within a few percent. Approximating the chamber as a right cylinder, the CO₂ flux, F ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$), was determined from the change in concentration as: $F = h \, dC/dt$, where h (cm) is the height from the top of the chamber to the soil surface. In addition to the height of the chamber segments (54 or 108 cm), we included the distance to the soil surface from the bottom of the chamber which was measured periodically for each mesocosm.

Leaf gas exchange

Leaf photosynthesis and stomatal conductance were taken on the youngest fully expanded leaf of *Avena barbata* plants in the mixed and grass monoculture communities and *Erodium botrys* plants in the forb monoculture communities. Photosynthesis and stomatal conductance were measured using a portable photosynthesis analyzer (Licor 6400, Licor Environmental). Gas exchange was measured at a PPFD of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (using the LI-COR 6400 LED light source) at ambient temperature and humidity. Baseline leaf and reference chamber CO₂ concentrations of $375 \mu\text{mol mol}^{-1}$ were achieved using a LI-COR 6400 CO₂ mixer. Measurements were initiated by sealing the leaf in the chamber. When CO₂ concentrations in the leaf chamber reached a steady state (60–90 s), leaf gas exchange values were logged.

Table 1 *F*-values from repeated measures ANOVA model testing the main effects and two-way interactions on whole ecosystem and leaf level gas exchange, canopy growth (height) and structure (LAI) and soil moisture content over time

Source of variance	df	NEE	R_e	ET	A_{max}	Canopy Ht	LAI	Soil moisture (10 cm)
Ppt	2	16.13**	38.91***	0.91	5.44*	22.86***	4.75*	12.65**
Dry	1	16.90*	0.92	0.27	7.28*	6.43	7.32*	178.66***
Veg	2	6.12*	1.44	4.21	34.13***	136.30***	5.33*	1.33
Time	4	3.55	20.99***	10.97***	4.40*	112.41***	85.04***	24.21***
Ppt × Dry	2	1.73	5.85*	2.57	9.38**	5.50*	0.77	0.84
Ppt × Veg	4	0.42	1.69	0.77	0.63	1.44	0.96	1.58
Ppt × Time	8	3.02*	1.07	3.70***	0.52	4.73***	0.87	10.17***
Dry × Veg	2	0.75	0.02	0.54	6.41*	1.06	2.12	0.62
Dry × Time	4	3.66*	3.02*	0.52	6.70**	7.65*	31.51**	2.99*
Veg × Time	8	7.07***	12.72***	0.77	4.91***	5.23***	5.78***	4.77***

The model includes data from cycles 2–6 but excludes the N addition plots. Ppt, cumulative precipitation treatment; Dry, difference in measured response 7 days into the dry period relative to the soil moisture maximum during the watering period; Veg, the three unique vegetation communities.

Significance designated as * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

ET, evapotranspiration; NEE, net ecosystem CO_2 exchange; N, nitrogen.

Measures of growth and canopy structure

Over the course of the experiment (water cycles 3–8), average canopy height was estimated by placing a measuring stick in the center of the mesocosm and determining average plant height by eye. LAI was estimated by averaging two measurements taken perpendicular to each other 5 cm above soil level using a ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, WA, USA).

Statistical analysis

Statistical analyses focused on the 2006–2007 growing season. Repeated measures analysis of variance (ANOVA) was used to test the main and interactive effects of treatment conditions on gas flux rates, canopy height, LAI and soil moisture (10 cm depth) using time as the ‘within’ factor (Gumpertz & Brownie, 1993). We chose to focus on soil moisture at 10 cm soil depth because the majority of the roots were located within this zone (data not presented). The first ANOVA model incorporated plant community composition (Veg), growing season cumulative precipitation (Ppt), and measured differences during the wet and dry periods (Dry) within the 21 day water cycle as fixed effects with repeated measures within a mesocosm over water cycles 2–6. Block was included in the model as a random effect. The second statistical model was similar to the first except that we analyzed data from cycles 4–6 (following the N applications) allowing us to include the effects of N (Fert) as an additional independent variable. For NEE, light intensity (PPFD) was included in both models as a

covariate. Pearson’s correlation and a stepwise multiple regression model were used to characterize relationships among dependent variables. Statistical significance was defined as $\alpha \leq 0.05$. Dependent variables were tested for normality and homogeneity of variance using Shapiro–Wilk *W* statistics to determine the goodness of fit of the data in normal quantile plots. A Box–Cox transformation was applied to the R_e and ET data to satisfy the assumptions of normality. All other data were found to be normally distributed. Statistical analysis was performed using SAS (version 9.1) and JMP (version 7.0) statistical software (SAS institute, Cary, NC, USA).

Results

Soil moisture

Trends in soil moisture did not differ significantly among the three plant community types (Table 1, Veg). Average SMC at 10 cm depth was 37% and 57% higher in ambient and high precipitation plots than low precipitation plots (Fig. 2). SMC changes over the course of the growing season varied among the precipitation treatments (Table 1, Ppt × Time), with soil moisture gradually declining through cycle 6 in the low precipitation plots while being maintained in ambient and high precipitation plots (Fig. 2). The SMC consistently peaked at the end of each watering period and then decreased 30–60% by the end of each soil drying period (Fig. 2), resulting in a strongly significant soil drying effect (Table 1, Dry). N addition significantly

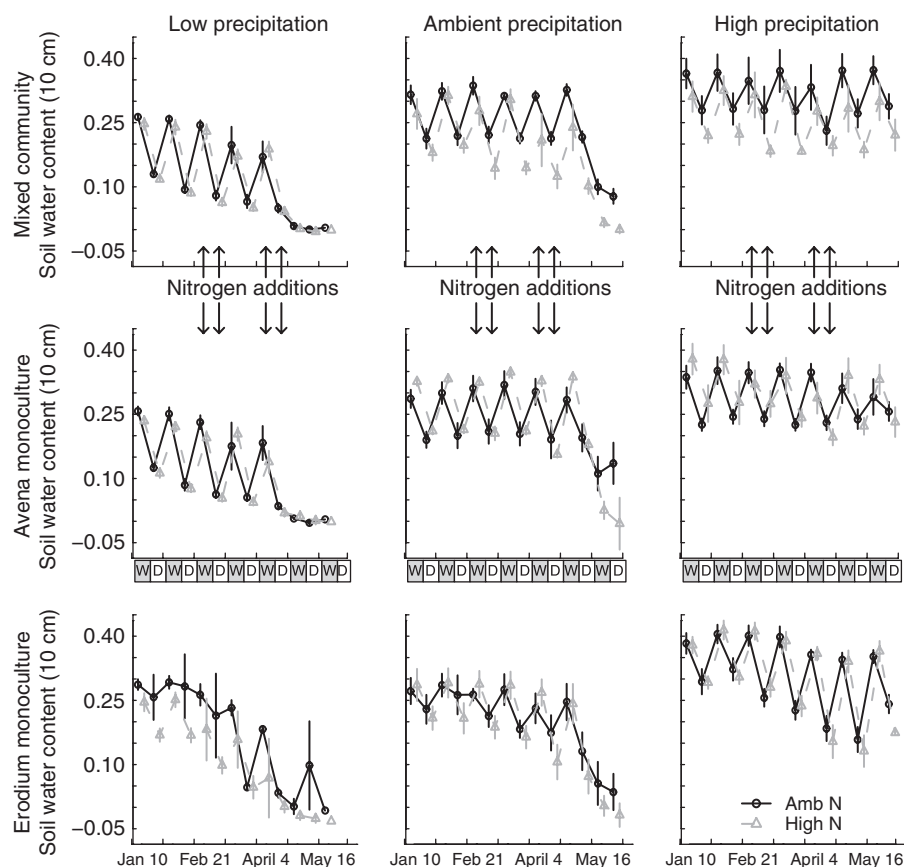


Fig. 2 Soil moisture (10 cm depth) changes in response to treatment conditions during the 2006–2007 season. The 'W' and 'D' on the X-axis in the center of the plot indicate the wet and dry period of each cycle. Cycles 2–8 are shown in the figures. The solid black line represents data from the ambient N plots and the dashed gray line indicates data points corresponding to N addition plots. Arrows indicate the four time points at which N additions was added. Symbols indicate mean values with the error bars representing SE.

influenced SMC (Table 2, Fert). A trend emerged in which N amendments promoted greater soil drying in the mixed plant community plots than the monoculture communities (Table 2, Fert \times Veg), particularly under ambient and high precipitation conditions (Fig. 2).

Ecosystem CO₂ and H₂O exchange

Ambient and high precipitation plots had NEE and dark respiration rates (R_e) that were significantly greater (Table 1, Ppt) than the low precipitation plots (Figs 3 and 4). However, precipitation-mediated differences in NEE and R_e averaged over cycles 2–6 were modest (+10–17.1%). As the growing season progressed, the low precipitation plots showed greater reduction in rates of NEE and ET relative to the ambient and high precipitation treatments as indicated by the significant Ppt \times Time interaction (Table 1, Figs 3 and 5). The soil drying periods (Dry) significantly decreased NEE on average by 18% over cycles 2–6, but did not significantly affect R_e or ET (Table 1, Figs 3–5). Ecosystem dark

respiration in low precipitation plots showed greater sensitivity to soil drying events (Fig. 4) than ambient or high precipitation plots (Table 1, Ppt \times Dry). A similar significant effect was seen for NEE and ET (Figs 3 and 5) during cycles 4–6 (Table 2, Ppt \times Dry).

Erodium monoculture plots had significantly lower NEE than either of the grass dominated communities, but there were no significant effects of vegetation type on R_e and ET (Table 1, Veg). NEE remained relatively stable over time, while R_e and ET tended to increase (Figs 3–5) as the season progressed (Table 1, Time). The majority of significant interaction terms in the first ANOVA model were interactions of the main effects with the time variable (Table 1).

N addition significantly increased NEE and R_e (Figs 3 and 4), an effect augmented by greater water availability (Table 2, Ppt \times Fert). Fertilization effects on NEE varied significantly among the three community types (Fig. 3) but showed no significant effects on R_e or ET (Table 2, Fert \times Veg). Fertilization effects on NEE and R_e tended to increase with time (Table 2, Fert \times Time).

Table 2 *F*-values from repeated measures ANOVA model testing the main effects and two-way interactions on ecosystem and leaf level gas exchange, canopy growth and structure and soil moisture content over time

Source of variance	df	NEE	R_e	ET	A_{max}	Canopy Ht	LAI	Soil moisture (10 cm)
Ppt	2	64.71***	86.58***	3.22	11.73**	85.46***	3.10	114.21***
Dry	1	86.80***	2.97	1.07	58.58***	18.99*	30.19**	486.49***
Fert	1	34.63***	82.73**	0.27	54.30***	11.10*	4.99	8.34*
Veg	2	0.52	8.61**	5.78*	149.17**	26.17***	9.04**	0.09
Time	2	3.22	11.53***	6.81*	3.68	411.94***	14.41**	23.84***
Ppt × Dry	2	15.23***	8.25***	6.66**	29.32***	4.04*	0.35	0.22
Ppt × Fert	2	11.59***	7.76***	0.40	0.47	21.99***	1.42	0.59
Ppt × Veg	4	1.69	5.42***	1.67	1.29	4.33	2.3	5.13***
Ppt × Time	4	8.12***	4.77***	1.42	1.93	14.21***	0.54	1.70
Dry × Fert	1	0.90	0.96	0.60	3.57	1.24	2.50	0.79
Dry × Veg	4	3.74*	0.37	1.56	7.35***	0.82	2.20	0.25
Dry × Time	2	27.50***	30.81***	1.46	7.91***	27.08***	63.69***	0.69
Fert × Veg	2	3.20*	0.06	1.00	1.77	0.30	2.33	4.34*
Fert × Time	2	6.17**	7.23***	0.18	1.94	3.60*	1.51	0.91
Veg × Time	4	3.07*	15.69***	0.19	38.49***	1.94	5.43***	2.79*

The model includes data from cycles 4–6 which includes the N treatment plots (Fert). Significance designated as * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. ET, evapotranspiration; LAI, leaf area index; NEE, net ecosystem CO₂ exchange.

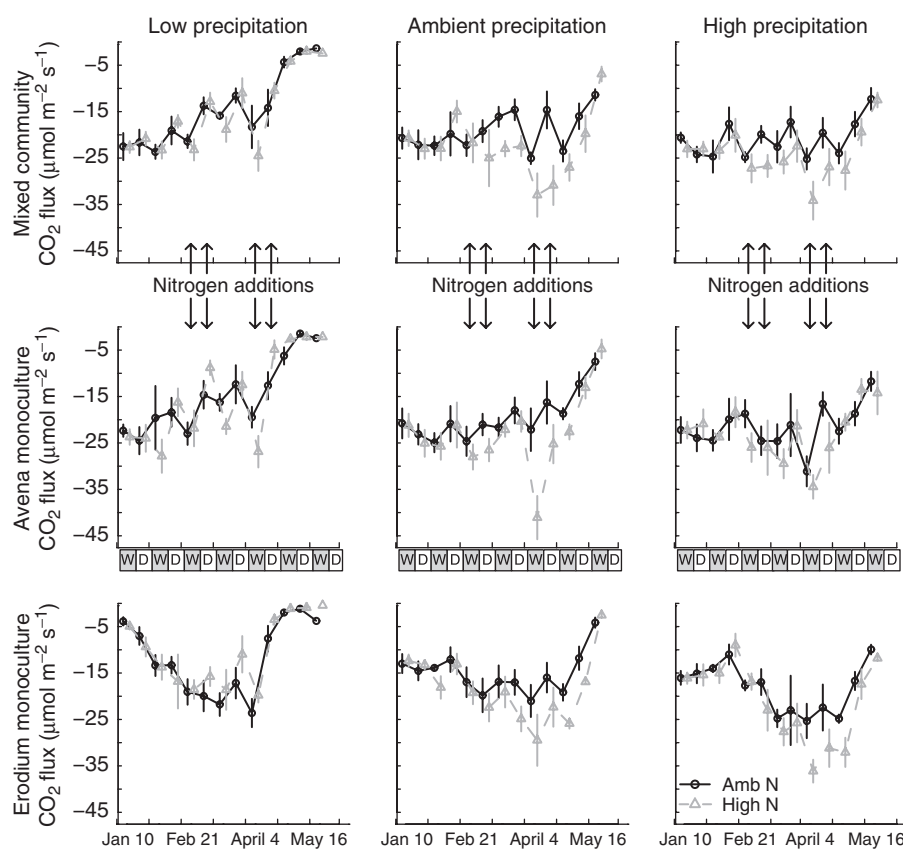


Fig. 3 Net ecosystem CO₂ exchange (NEE) in response to treatment conditions over the course of the 2006–2007 season. The 'W' and 'D' on the X-axis in the center of the plot indicate the wet and dry period of each cycle. By standard convention more negative values represent greater ecosystem CO₂ fixation rates. Symbols indicate mean values with the error bars representing SE.

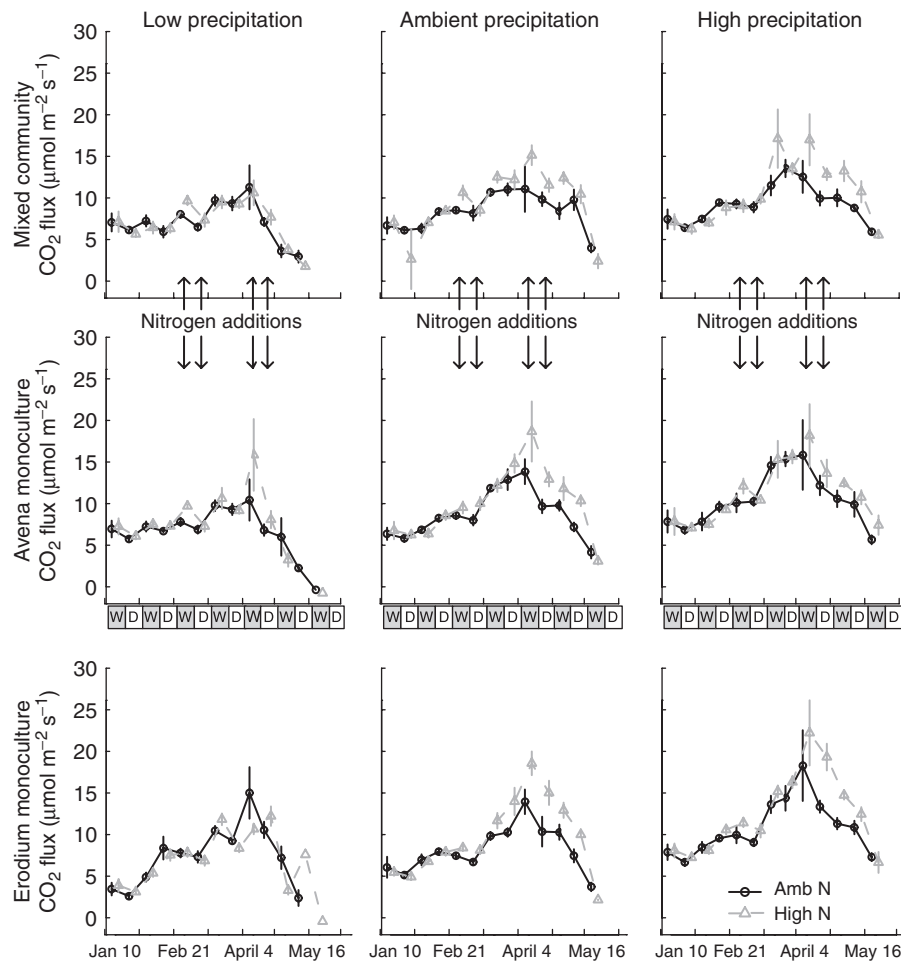


Fig. 4 Ecosystem respiration (R_e) in response to treatment conditions over the 2006–2007 season. The 'W' and 'D' on the X-axis in the center of the plot indicate the wet and dry period of each cycle. Symbols indicate mean values with the error bars representing SE.

NEE was most strongly correlated with leaf photosynthesis, canopy height, PPFD and SMC at 25 cm (Table 3). Those four factors explained 38% of the variation in NEE data in the multiple regression model. Ecosystem dark respiration was most strongly correlated with canopy height and LAI (Table 3), with canopy height, LAI, PPFD and SMC explaining 31% of the variation in the multiple regression model. Ecosystem ET was most strongly correlated with canopy height and PPFD, with those two factors explaining 39% of the variation in the multiple regression analysis.

Leaf gas exchange

The main effects of precipitation and soil drying on leaf photosynthesis were statistically significant (Table 1, Ppt \times Dry). The sensitivity of leaf photosynthesis to soil drying was greatest in low precipitation plots (Table 1, Ppt \times Dry). *Erodium* had higher rates of photosynthesis than *Avena* (Fig. 6) growing in mixed or monoculture

communities (Table 1, Veg). The effects of both soil drying and vegetation type on photosynthesis did change significantly over time (Table 1). N addition significantly stimulated leaf photosynthesis but did not interact significantly with any of the other treatment factors (Table 2, Fert). Leaf photosynthesis was most strongly correlated with SMC (Table 3). Stomatal conductance was strongly correlated with photosynthesis as expected, but was not significantly correlated with measures of ecosystem CO_2 and water vapor exchange (Table 3).

Canopy height and LAI

Average heights of mixed and monoculture grass community (both 41 cm) were significantly greater (Table 1, Veg) than the *Erodium* monoculture community (31 cm). Vegetation in ambient and high precipitation plots was significantly taller (Table 1, Ppt) than vegetation in low precipitation plots (42 and 44 vs. 34 cm). N addition significantly increased canopy height by 10% (Table 2,

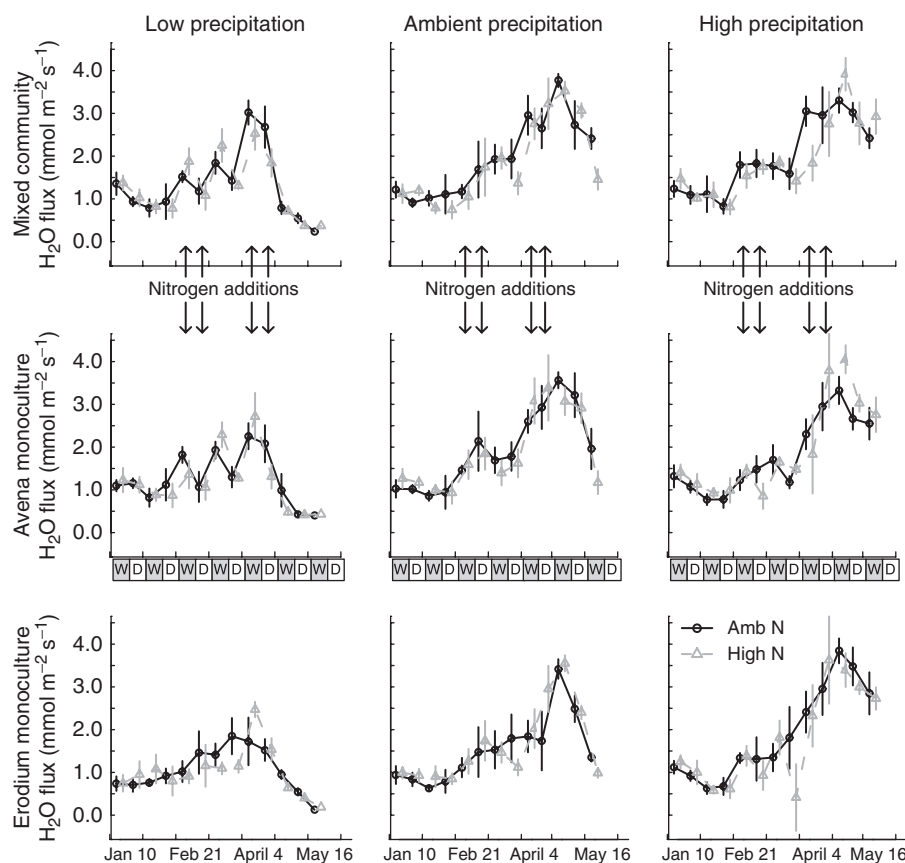


Fig. 5 Ecosystem evapotranspiration rates in response to treatment conditions over the 2006–2007 season. The ‘W’ and ‘D’ on the X-axis in the center of the plot indicate the wet and dry period of each cycle. Symbols indicate mean values with the error bars representing SE.

Table 3 Pearson’s correlation matrix indicating the relationship between dependent variables in the experiment

	NEE	R_e	ET	A_{max}
Leaf photosynthesis	0.33***	0.20***	0.01	
Leaf stomatal conductance	0.01	−0.15**	−0.35***	0.80***
Leaf transpiration	0.15***	0.04	0.10***	0.36***
Canopy height	0.29***	0.42***	0.50***	0.14**
LAI	0.13**	0.38***	0.20***	0.13**
PPFD	0.35***	0.28***	0.57***	0.12***
SMC 10 cm	0.25***	0.13***	0.08*	0.36***
SMC 25 cm	0.33**	0.19***	0.13***	0.42***
SMC 50 cm	0.20***	0.13***	0.08*	0.34***
Soil Tm 3 cm	0.06	0.44***	0.39**	0.27*
Soil Tm 10 cm	0.02	0.44***	0.34**	0.29*
Soil Tm 25 cm	0.03	0.41***	0.35**	0.42***

Correlative relationships are based on data collected during cycles 4–6 during the 2007 season. SMC, soil moisture content at depths of 10, 25 and 50 cm. Soil Tm, soil temperature at 3, 10 and 25 cm; LAI, leaf area index and PPFD, photosynthetic photon flux density. Significance designated as

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. ET, evapotranspiration; NEE, net ecosystem CO_2 exchange.

Fert) and positive growth responses to N addition increased with greater water availability (Table 2, Ppt \times Fert).

The mixed vegetation community had a significantly higher LAI (Table 1, Veg) than monoculture communities (4.5 vs. 3.5). There was a significant trend of greater LAI with increasing precipitation (Table 1, Ppt), but the range across precipitation treatments was only 4.0–4.2. N additions had no significant influence on LAI (Table 2, Fert).

Discussion

Our results highlight the importance of considering the interactive effects that precipitation patterns and N availability have on leaf and ecosystem CO_2 exchange and ET. When combined, changes in season precipitation totals, soil drying, N availability and plant community composition influence CO_2 and water vapor fluxes in ways that could not have been predicted from the manipulation of any single variable alone. The results generally support our first two hypotheses that gas exchange rates respond positively to greater water

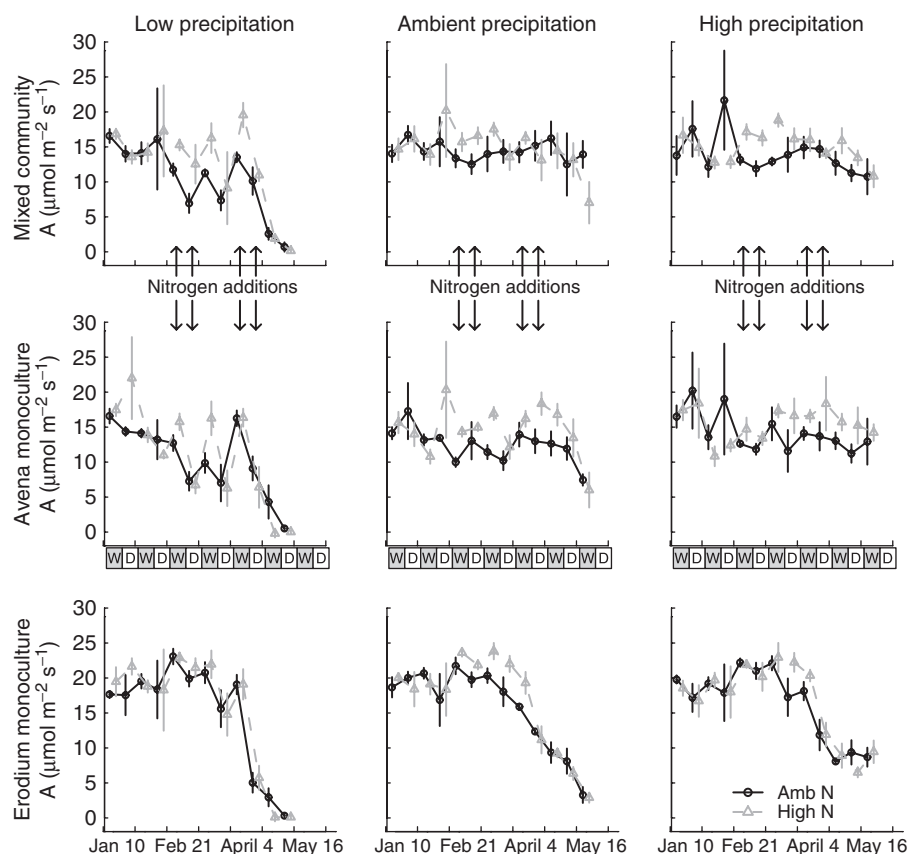


Fig. 6 Leaf level photosynthesis responses to treatment conditions over the 2006–2007 season. The ‘W’ and ‘D’ on the X-axis in the center of the plot indicate the wet and dry period of each cycle. Symbols indicate mean values with the error bars representing SE.

availability and show sensitivity to intermittent periods of water deficit. Greater water availability increased NEE, R_e and canopy height responsiveness to N amendments, as outlined in the third hypothesis. Our prediction that plant community composition would have a strong influence on response to treatment conditions, was generally not supported, as all three plant communities tended to respond similarly to precipitation, drying events and N treatments. These results suggest that the effects of water and N availability may be more generalizable across grassland community diversity than we initially predicted.

In semiarid grasslands, water pulses can strongly affect whole ecosystem and leaf level gas exchange (Huxman *et al.*, 2004), particularly following extended periods of water deficit (Potts *et al.*, 2006). Strong increases (50–100%) in NEE were observed in both Sonoran and Chihuahuan desert ecosystem by increasing water inputs by 25–50% during the monsoon season (Potts *et al.*, 2006; Patrick *et al.*, 2007). Harpole *et al.* (2007a, b) showed similar increases in NEE, R_e and ET at the end of the growing season in response to 30% increases in water inputs over the growing season. Risch & Frank (2006) showed much lower

NEE stimulation (1.6–11.5%) in response to a 50% increase in water inputs in grassland systems in Yellowstone National Park. Season-integrated NEE, R_e and ET responses to increased water availability were relatively modest (10–17%) compared with studies conducted in the more arid ecosystems. It is evident, however, that the precipitation effect became more pronounced (Fig. 3) as the season progressed (Table 1, Ppt \times Time). For example, by cycle 6, NEE and R_e in ambient and high precipitation plots were $\sim 35\%$ higher compared with low precipitation plots, which was much greater than the integrated average over cycles 2–6. This suggests that differences in cumulative growing season precipitation have their strongest influence on ecosystem fluxes later in the season (March–May). The significant effect of time dependent factors on ecosystem CO_2 and H_2O fluxes observed in this study suggests that snapshot measurements of gas fluxes may not always be representative of season long trends.

Late season shifts in ecosystem CO_2 and H_2O flux sensitivity to precipitation patterns are likely driven by both changes in soil moisture status and biomass accumulation. For example, rising vapor pressure deficit from winter to spring and increasing plant biomass will

tend to increase soil moisture losses through ET. The significant precipitation by time interaction (Table 1) for soil moisture at 10 cm indicates a general decrease in soil moisture as the season progressed, an effect most pronounced in the low precipitation plots (Fig. 2). Greater soil moisture may also increase ecosystem flux rates by stimulating plant growth, resulting in increasing leaf area as the season progresses. Canopy height, which increased concomitantly with greater water availability in this study, explained a significant amount of the variation in ecosystem CO_2 and H_2O fluxes (Table 3).

Flux rates in our more mesic study system were greater than those seen in sparsely vegetated desert ecosystems where water deficit is more extreme (Potts *et al.*, 2006; Patrick *et al.*, 2007). These higher flux rates are partially a function of greater water availability which increases physiological function and tends to produce denser grass communities (Pitt & Heady, 1978). In this study, plant canopy height and LAI, increased significantly in response to greater water availability which tends to increase ecosystem CO_2 and H_2O exchange capacity. However, increases in plant biomass and density in response to the alleviation of below ground resource constraints (e.g. water and N) will at a certain point result in competition for light, which can constrain whole canopy photosynthesis (Lane *et al.*, 2000). In contrast, competition for light is rare in arid ecosystems with low net primary productivity (Kicklighter *et al.*, 1999). This may partially explain why ecosystem flux rates increased modestly in response to greater water availability in our system compared with arid desert ecosystems.

We anticipated that higher precipitation plots would be less sensitive to the soil drying periods and that sensitivity would increase later in the season as biomass increased. Among the most intriguing results of this study is that the soil drying period within each water cycle consistently depressed NEE across the range of precipitation totals and plant community types in the study (Table 1, Dry). This suggests that NEE in grassland systems may be poorly buffered against periods of drought even in years of high cumulative moisture. Interestingly, R_e and ET showed much less sensitivity to these soil drying periods (Table 1, Dry), indicating that the potential for carbon storage in these grassland communities may decrease significantly during periods of water deficit. During the soil drying period, ambient and high precipitation plots consistently had SMCs between 20% and 30%, while low precipitation plots were between 10% and 20% (Fig. 2). The observed decreases in NEE in the higher precipitation plots in response to soil drying suggest that the soil moisture optimum for maximum NEE is $>20\%$ in our study system. Overall, our data is consistent with other stu-

dies, which suggest that interannual precipitation events (e.g. soil drying events) can have equal or greater effects on grassland productivity and gas exchange than annual precipitation totals (Baldocchi *et al.*, 2004; Emmerich & Verdugo, 2008; Robertson *et al.*, 2009).

A previous study in a California annual grassland showed that late season carbon fluxes were negatively affected by N amendments (Harpole *et al.*, 2007b). In contrast, we found that N addition stimulated NEE, R_e and A_{max} (Figs 3, 4 and 6) and that higher precipitation totals increased the positive effect of N availability on NEE and R_e (Table 2, Ppt \times Fert). Reduced SMC can limit N transport to roots through reductions in rates of mass flow (Barber, 1995). Soil moisture conditions also have important impacts on soil microbial community activity and their ability to contribute to the soil bioavailable N pool (Stark & Firestone, 1995; Bell *et al.*, 2008). N addition significantly stimulated both height growth and aboveground biomass (data not presented), which is consistent with N amendment effects in other California annual grassland studies (Dukes *et al.*, 2005). This suggests that increased carbon fluxes in response to N could partially be the result of growth stimulation that resulted in greater leaf area. Leaf level photosynthesis increased significantly in response to N addition (Fig. 6), indicating that increases in NEE may also have been related to stimulation of photosynthesis through increased N availability. The N treatment significantly increased foliar N concentrations, which supports this interpretation (data not presented).

Soil moisture and N availability can alter the carbon and water exchange capacity of grasslands via changes in leaf physiology and canopy structure. Stomatal closure under soil moisture deficit (Novick *et al.*, 2004) and suboptimal leaf N content (Flanagan *et al.*, 2002; Risch & Frank, 2006) are important constraints to carbon exchange in grassland systems. Water and N availability can also alter canopy structure either through changes in plant density, growth rates or shifts in community composition (Zavaleta *et al.*, 2003), each of which can strongly modulate light capture and ecosystem carbon and water exchange capacity (Baldocchi *et al.*, 2004; Ivans *et al.*, 2006; Risch & Frank, 2006). In this study, leaf photosynthesis and canopy traits (height and LAI) exhibited sensitivity to cumulative water status, soil drying and N availability (Table 1), but that translated to only moderate correlations with ecosystem CO_2 and H_2O fluxes (Table 3). This demonstrates the complexity of the relationship between soil resource status and its effects on ecosystem CO_2 and H_2O gas exchange. While the three communities types studied were inherently different in canopy structure and height, their response to water conditions did not vary (Table 1, Ppt \times Veg and Dry \times Veg). This result suggests

that functional responses to changes in water conditions may be similar across a range of grassland community types, even when they differ widely in structural traits (rooting depth, LAI, and other measures).

Conclusion

Our study shows that periods of soil moisture deficit, which are projected to increase in subtropical regions under future climate scenarios, tend to reduce carbon exchange capacity over a range of cumulative precipitation totals and annual grassland community types. The results suggest that even high annual precipitation will not offset reductions caused by periods of droughts. N stimulation of NEE observed in this study suggests it increases carbon storage capacity into biomass, particularly in conjunction with higher annual precipitation totals. However, some of the increases in carbon fixing capacity expected to occur with greater NEE may be partially offset by higher R_e . Ecosystem water vapor exchange generally showed less sensitivity to water and N availability, suggesting that it may be more buffered than carbon exchange.

Further research is needed to determine whether the soil drying pulse effects observed here ultimately lead to more or less cumulative carbon fixation capacity within an ecosystem. It is clear, however, that the timing of moisture may be just as important as annual precipitation total in influencing rates of carbon fixation. Importantly, our results show that responsiveness of ecosystem CO_2 exchange to patterns of water and N availability are relatively consistent over the range of grassland plant community diversity assessed in this study.

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References

Baldocchi DD, Xu LK, Kiang N (2004) How plant functional-type, weather, seasonal drought, and soil physical properties alter water and energy fluxes of an oak-grass savanna and an annual grassland. *Agricultural and Forest Meteorology*, **123**, 13–39.

Barber SA (1995) *Soil Nutrient Bioavailability: A Mechanistic Approach*. John Wiley & Sons, New York.

Bell C, McIntyre N, Cox S, Tissue D, Zak J (2008) Soil microbial responses to temporal variations of moisture and temperature in a Chihuahuan Desert Grassland. *Microbial Ecology*, **56**, 153–167.

Calanca P, Roesch A, Jasper K, Wild M (2006) Global warming and the summertime evapotranspiration regime of the Alpine region. *Climatic Change*, **79**, 65–78.

Corbin J, Dyer AR, Seabloom EW (2007) Competitive interactions. In: *Ecology and Management of California Grasslands* (eds D'Antonio C, Corbin J, Stromberg M), pp. 156–168. University of California Press, Berkeley.

Dukes JS, Chiariello NR, Cleland EE *et al.* (2005) Responses of grassland production to single and multiple global environmental changes. *PLOS Biology*, **3**, 1829–1837.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling, and impacts. *Science*, **289**, 2068–2074.

Emmerich WE, Verdugo CL (2008) Precipitation thresholds for CO_2 uptake in grass and shrub plant communities on walnut gulch experimental watershed. *Water Resources Research*, **44**, W05S16, doi: 10.1029/2006WR005693.

Fenn ME, Baron JS, Allen EB *et al.* (2003a) Ecological effects of nitrogen deposition in the western United States. *Bioscience*, **53**, 404–420.

Fenn ME, Haeuber R, Tonnesen GS *et al.* (2003b) Nitrogen emissions, deposition, and monitoring in the western United States. *Bioscience*, **53**, 391–403.

Field CB, Chapin FS, Chiariello NR, Holland EA, Mooney HA (1996) The jasper ridge CO_2 experiment: design and motivation. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 121–142. Academic Press, New York.

Flanagan LB, Wever LA, Carlson PJ (2002) Seasonal and interannual variation in carbon dioxide exchange and carbon balance in a northern temperate grassland. *Global Change Biology*, **8**, 599–615.

Galloway JN, Dentener FJ, Capone DG *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.

Groisman PY, Knight RW, Easterling DR, Karl TR, Hegerl GC, Razuvaev VAN (2005) Trends in intense precipitation in the climate record. *Journal of Climate*, **18**, 1326–1350.

Gumpertz ML, Brownie C (1993) Repeated measures in randomized block and split-plot experiments. *Canadian Journal of Forest Research*, **23**, 625–639.

Gurevitch J, Scheiner SM, Fox GA (2006) *The Ecology of Plants*. Sinauer, Sunderland.

Harpole WS, Goldstein L, Aicher R (2007a) Resource limitation. In: *Ecology and Management of California Grasslands* (eds D'Antonio C, Corbin J, Stromberg M), pp. 119–127. University of California Press, Berkeley.

Harpole WS, Potts DL, Suding KN (2007b) Ecosystem responses to water and nitrogen amendment in a California grassland. *Global Change Biology*, **13**, 2341–2348.

Harte J, Torn MS, Chang FR, Feifarek B, Kinzig AP, Shaw R, Shen K (1995) Global warming and soil microclimate - results from a meadow-warming experiment. *Ecological Applications*, **5**, 132–150.

Hayhoe K, Cayan D, Field CB *et al.* (2004) Emissions pathways, climate change, and impacts on California. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 12422–12427.

Huxman TE, Snyder KA, Tissue D *et al.* (2004) Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia*, **141**, 254–268.

- IPCC (2007) *Working Group 1 Fourth Assessment Report*. Cambridge University Press, Cambridge.
- Ivans S, Hipps L, Leffler AJ, Ivans CY (2006) Response of water vapor and CO₂ fluxes in semiarid lands to seasonal and intermittent precipitation pulses. *Journal of Hydrometeorology*, **7**, 995–1010.
- Kicklighter DW, Bondeau A, Schloss AL, Kaduk J, McGuire AD (1999) Comparing global models of terrestrial net primary productivity (NPP): global pattern and differentiation by major biomes. *Global Change Biology*, **5**, 16–24.
- Knapp AK, Fay PA, Blair JM *et al.* (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, **298**, 2202–2205.
- Knapp AK, Smith MD (2001) Variation among biomes in temporal dynamics of aboveground primary production. *Science*, **291**, 481–484.
- Lane DR, Coffin DP, Lauenroth WK (2000) Changes in grassland canopy structure across a precipitation gradient. *Journal of Vegetation Science*, **11**, 359–368.
- LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.
- Nemani RR, Keeling CD, Hashimoto H *et al.* (2003) Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science*, **300**, 1560–1563.
- Novick KA, Stoy PC, Katul GG, Ellsworth DS, Siqueira MBS, Juang J, Oren R (2004) Carbon dioxide and water vapor exchange in a warm temperate grassland. *Oecologia*, **138**, 259–274.
- Patrick L, Cable J, Potts D *et al.* (2007) Effects of an increase in summer precipitation on leaf, soil, and ecosystem fluxes of CO₂ and H₂O in a sotol grassland in Big Bend National Park, Texas. *Oecologia*, **151**, 704–718.
- Pitt MD, Heady HF (1978) Responses of annual vegetation to temperature and rainfall patterns in northern California. *Ecology*, **59**, 336–350.
- Poorter H, Perez-Soba M (2001) The growth response of plants to elevated CO₂ under non-optimal environmental conditions. *Oecologia*, **129**, 1–20.
- Potts DL, Huxman TE, Cable JM *et al.* (2006) Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. *New Phytologist*, **170**, 849–860.
- Risch AC, Frank DA (2006) Carbon dioxide fluxes in a spatially and temporally heterogeneous temperate grassland. *Oecologia*, **147**, 291–302.
- Robertson TR, Bell CW, Zak JC, Tissue DT (2009) Precipitation timing and magnitude differentially affect aboveground annual net primary productivity in three perennial species in a Chihuahuan Desert grassland. *New Phytologist*, **181**, 230–242.
- Saleska SR, Harte J, Torn MS (1999) The effect of experimental ecosystem warming on CO₂ fluxes in a montane meadow. *Global Change Biology*, **5**, 125–141.
- Schimel DS, Braswell BH, Parton WJ (1997) Equilibration of the terrestrial water, nitrogen, and carbon cycles. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 8280–8283.
- Stark JM, Firestone MK (1995) Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, **61**, 218–221.
- Sun Y, Solomon S, Dai AG, Portmann RW (2007) How often will it rain? *Journal of Climate*, **20**, 4801–4818.
- Suttle KB, Thomsen MA, Power ME (2007) Species interactions reverse grassland responses to changing climate. *Science*, **315**, 640–642.
- Vitousek P (2004) *Nutrient Cycling and Limitation: Hawaii as a Model System*. Princeton University Press, Princeton.
- Vitousek PM, Aber JD, Howarth RW *et al.* (1997a) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.
- Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997b) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.
- Weltzin JF, Loik ME, Schwinning S *et al.* (2003) Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience*, **53**, 941–952.
- Zavaleta ES, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, Field CB, Mooney HA (2003) Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs*, **73**, 585–604.